



Growth performance of *Labeo bata* (Hamilton, 1822) in freshwater and its acclimatization in brackish water with betaine as feed additive

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ARTICLE INFO

Keywords:

Labeo bata
Betaine
Feed
Growth
Salinity
Toxicity
Resistance

ABSTRACT

Climate change is one of the major causes of coastal flood and tropical cyclone which results in salinity changes in large scale coastal area. Salinity affects survival and growth of many aquatic organisms and causes stress of freshwater fish affecting fisheries in various parts of the world. However, incorporation of 'Betaine' in the supplementary feed may be way out for freshwater fish species grown in saline environment. In this study, the experiments were conducted with *Labeo bata*, a freshwater fish species with two feeds, T0 (non-betaine feed) and T1 (betaine added feed) in three phases: (i) growth study for 50 days in freshwater, (ii) toxicity test on salinity for 96 h, with loss of body weight and muscle tissue water, and (iii) salinity resistance test for 12 days in gradually increasing salinities @ 1.0 ppt day⁻¹. Results showed that growth rate (%) was significantly ($p < 0.05$) higher in betaine fed fish (16.79 ± 0.84 – 27.91 ± 3.33) as compared to non-betaine fed fish (13.58 ± 1.25 – 15.36 ± 0.29) throughout the experimental period. Betaine fed fish showed no mortality even at 12 ppt after 96 h exposure whereas all non-betaine fed fish showed 100% mortality within 24 h at 12 ppt. Over all, non-betaine fed fish lost 10.3% higher amount of water in muscle tissue as compared to that in betaine fed fish while grown in saline condition. The beneficial effect of betaine as feed additive described in this study may encourage farmers to culture *L. bata* up to 12 ppt.

1. Introduction

Changing climate is predicted to cause sea level rise (IPCC, 2007, 2013) which, in turn, may affect a large scale area to be saline, resulting in the less amount of surface freshwater particularly in coastal region. The saline environment is responsible for salinity mediated water stress of freshwater fisheries in various parts of the world (Cruz et al., 2007; Badjeck et al., 2010). In tropics, fluctuations of salinity are very pronounced where the climate is characterized by wet and dry seasons (Suresh and Lin, 1992). Salinity is one of the important environmental factors affecting survival, growth and distribution of many aquatic organisms (Kumlu et al., 1999, 2000). It can change the amount of energy available for growth of fishes by altering the energetic cost of ionic and osmotic regulation (Parry, 1966); however, the relation between salinity and growth is complex and not readily predicted (Morgan and Iwama, 1996). The effect of salinity on growth of stenohaline freshwater species appears to vary among species. Common carp, *Cyprinus carpio* L. had the fastest growth in 0.5 ppt salinity (growth rate was not determined in freshwater) and growth rate decreased with increasing salinity to 10.5 ppt (Wang et al., 1997). However, growth of bluegill,

Lepomis macrochirus was not affected by salinity ranging from 0.5–10 ppt (Musselman et al., 1995).

In West Bengal, India, many areas in Sundarban delta (UNESCO declared World Heritage Site) are vulnerable to saline water inundation and subjected to environmental hazard during extreme weather conditions like cyclones and storm surges. In 2009, the severe tropical cyclone 'Aila' struck the Sundarban, inundating extensive freshwater areas mixed with brackish water. Even, it brought changes the parameter of brackish water that salinity increased from 14 ppt to 17.5 ppt with an increase of 25% (Mitra et al., 2011). Due to salinity intrusion in freshwater aquaculture areas, many freshwater species were subjected to severe salinity stress and some species perished due to their inability to cope up with such extreme conditions (Mitra et al., 2011).

Labeo bata, a freshwater carp, is considered as an important candidate species in aquafarming and cultured widely because of certain advantages such as good taste, consumer's preference, high market demand, good growth rate, thriving well in shallow waters, omnivorous feeding habit, and acceptability to artificial feed. It is widely cultured in freshwater bheries in coastal region of the Sunderban areas, West Bengal. To acclimatize this species in a low saline environment, betaine,

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<https://doi.org/10.1016/j.aquaculture.2018.11.020>

Received 28 June 2018; Received in revised form 10 November 2018; Accepted 10 November 2018

Available online 11 November 2018

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known as trimethylglycine (TMG) as hydrochloride form, is added in supplementary feed as an additive. Selection of betaine as a feed additive is considered due to the specific reason. It is a methyl derivative of non-toxic amino acid glycine (Kettunen et al., 2001), and belongs to a small group of chemicals $\{(\text{CH}_3)_3\text{N}^+ \text{CH}_2\text{COO}^-\}$ such as free amino acids, nucleotides, nucleosides and quaternary ammonium base (Takea and Takii, 1992; Penafiora and Virtanen, 1996; Dias et al., 1997; Papatryphon and Soares, 2000). It is also known to stimulate the olfactory bulb of fish and accumulates in muscle cells to benefit fish growing in changing salinities (Clarke et al., 1994; Castro et al., 1998). Besides, it has a major metabolic function to act as an osmolyte to reduce the consequences of osmotic stress in fish (Castro et al., 1998). The basic mechanism of the osmolyte function is an active uptake or synthesis of betaine in cells exposed to hyperosmotic stress and a consequent rise in cell osmolality to maintain the water balance. Because betaine is highly compatible with enzyme function, its accumulation has far fewer adverse effects on cell metabolism than the main inorganic intracellular ions (Bowling and Somero, 1979). With this backdrop, the present study was conducted to know the effect of betaine as a feed additive with two specific objectives: (i) growth performance of *Labeo bata* fed with betaine and non-betaine feeds in freshwater, and (ii) the degree of survival of freshwater grown *Labeo bata* fed with betaine and non-betaine feeds in saline environment.

2. Materials and methods

2.1. Experimental animals and their acclimatization

Healthy fingerlings of *Labeo bata* were obtained from private fish farm in Naihati, North-24 Parganas district of West Bengal, India during the end of January 2017. Fishes ($3.575 \pm 0.00 \text{ g}$ and $5.945 \pm 0.03 \text{ cm}$) were acclimatized to environmental condition (temperature, $20\text{--}27^\circ\text{C}$; pH, $7.5\text{--}8.0$; DO, $5.6\text{--}6.7 \text{ mg l}^{-1}$, CO_2 , $4.1\text{--}4.9 \text{ mg l}^{-1}$; alkalinity, $160\text{--}163 \text{ mg l}^{-1}$; hardness, $820\text{--}970 \text{ mg l}^{-1}$) for one week in circular FRP (fiberglass reinforced plastic) tanks ($r = 45.00 \text{ cm}$, $h = 95.00 \text{ cm}$), each filled up with 500 l freshwater. During acclimatization fish were fed with pelleted feed containing 30% protein @ 3% of body weight. Fecal matter and unutilized feed materials were siphoned out and 25–30% of water exchange was done with freshwater on every alternate day.

2.2. Formulation and proximate composition of experimental feeds

Two test feeds such as T0 and T1 were prepared, with the equal amount of same ingredients in $\text{g } 100 \text{ g}^{-1}$ of each feed (fish meal, 25; mustard oil cake, 32; rice bran, 18; soybean, 12; butylated hydroxytoluene (BHT), 0.01; and vitamin and mineral premix, 01), except wheat flour 11.99 g in T0 and 11.74 g in T1. The deficit 0.25 g of wheat flour in T1 was compensated with 0.25 g betaine to make 100 g . Table 1 shows the proximate composition of feed ingredients and formulated feeds. To analyze the proximate composition, the methods were followed: Crude protein was estimated by multiplying total nitrogen content of the sample with 6.25. Total nitrogen content of fish meal, mustard oil cake, rice bran, wheat flour and soybean meal was

estimated using Micro – Kjeldahl's method (Kjelplus) (AOAC, 2005). The crude fat of fish meal, mustard oil cake, rice bran, wheat flour and soybean meal was estimated using the Soxhlet extracting apparatus by extracting the fat content of the sample for 6–8 h with petroleum ether (boiling point $40\text{--}60^\circ\text{C}$) (AOAC, 2005). To determine ash content of fish meal, mustard oil cake, rice bran, wheat flour and soybean meal, the sample were burnt at $550^\circ\text{C} \pm 20^\circ\text{C}$ for 6 h in muffle furnace. Nitrogen Free Extract (NFE) of carbohydrate was determined following the method (Hastings, 1976): Nitrogen Free Extract (NFE %) = $100 - (\text{percent moisture} + \text{percent crude protein} + \text{percent crude fat} + \text{percent ash})$.

2.3. Preparation of artificial saline medium

Artificial saline water (35 ppt) was prepared with the following composition as (A + B + C); A. Gravimetric salts (g kg^{-1} of solution): NaCl, 23.926; Na_2SO_4 , 4.008; KCl, 0.677; NaHCO_3 , 0.196; KBr, 0.098; H_2BO_3 , 0.026; NaF, 0.003; B. Volumetric salts (Moles kg^{-1} of solution): $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.05327; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01033; $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, 0.00009; C. 1000 ml distilled water.

2.4. Experimental design

The entire experiment was conducted in three phases, each was tested with two different feeds (T0 and T1): (i) Growth study: fish were reared in freshwater for 50 days in FRP tanks. (ii) Toxicity test on salinity: fish were tested in graded levels of salinities such as 0 ppt (control), 3 ppt, 6 ppt, 9 ppt, and 12 ppt in glass aquaria for 96 h to determine Median Lethal Salinity ($\text{MLS}_{96 \text{ h}}$), with loss of body weight and muscle tissue water, and (iii) Salinity resistance test: fish were tested for their efficiency of salinity resistance in glass aquaria with gradually increasing salinity @ 1.0 ppt day^{-1} until it reached 12 ppt.

2.4.1. Growth study

Six FRP tanks (3 for T0 feed and 3 for T1 feed), each with 600 l capacity, were cleaned thoroughly using scrub and then dried for three days. Each tank was then filled with 500 l freshwater. Acclimatized fishes were segregated and stocked in FRP tanks @ 30 numbers tank $^{-1}$, corresponding to $4.72 \text{ lakhs ha}^{-1}$. Mean weight and length of fish for T0 feed was respectively $3.57 \pm 0.00 \text{ g}$ and $5.96 \pm 0.03 \text{ cm}$ and for T1 feed was respectively $3.58 \pm 0.00 \text{ g}$ and $5.93 \pm 0.03 \text{ cm}$. Feeds were applied in each experimental tank twice a day (10 AM and 16 PM) @ 3% of fish body weight. The tanks were covered with nylon net to prevent the jumping out of fishes. The experiment continued for 50 days.

2.4.2. Toxicity test on salinity

Toxicity test on salinity was done to determine Median Lethal Salinity for 96 h ($\text{MLS}_{96 \text{ h}}$): it is a measure of the lethal dose of salinity to kill half of the numbers or 50% of the tested population after 96 h. Glass aquaria (each with $60 \text{ cm} \times 30 \text{ cm} \times 30 \text{ cm}$; capacity 54 l) were filled up to 50 l with respective salinities (0 ppt, 3 ppt, 6 ppt, 9 ppt and 12 ppt), prepared by serial dilution from stock saline water. Then fish after 50 days of growth study were released @ 5 nos aquaria $^{-1}$ for

Table 1

Proximate composition of feed ingredients and formulated feeds.

Feed ingredients	Moisture (%) (Mean $\pm$ S.D)	Crude protein (%) (Mean $\pm$ S.D)	Crude fat (%) (Mean $\pm$ S.D)	Ash (%) (Mean $\pm$ S.D)	NFE (%) (Mean $\pm$ S.D)
Fish meal	9.49 ± 0.25	52.44 ± 1.45	6.84 ± 0.23	16.78 ± 0.68	14.45 ± 0.45
Wheat flour	6.04 ± 0.18	12.24 ± 0.45	4.47 ± 0.16	2.42 ± 0.15	74.83 ± 1.24
Rice bran	9.16 ± 0.28	6.53 ± 0.32	2.50 ± 0.12	14.94 ± 0.32	66.87 ± 0.90
Soybean	11.92 ± 0.36	44.60 ± 0.98	1.50 ± 0.09	14.46 ± 0.56	27.53 ± 0.56
Mustard oil cake	9.20 ± 0.5	30.80 ± 1.0	14.87 ± 0.21	9.70 ± 0.8	34.02 ± 0.75
Formulated diet (T0)	7.80 ± 0.32	30.62 ± 0.95	6.88 ± 0.26	10.80 ± 0.32	34.33 ± 1.32
Formulated diet (T1)	7.82 ± 0.35	30.62 ± 0.90	6.88 ± 0.25	11.00 ± 0.2	34.08 ± 1.33

toxicity test, each with triplicate. Mean weight and length of fish for T0 feed was 7.05 ± 0.03 g; 8.75 ± 0.03 cm and mean weight and length of fish for T1 feed was 9.50 ± 0.06 g; 9.07 ± 0.02 cm. The fish were fed with respective feeds (T0 feed for T0 treated fish and T1 feed for T1 treated fish) @ 3% of fish body weight during the experiment twice a day (10 AM and 16 PM). The fecal matter and left over feed were siphoned out daily and approx 20–25% water of respective salinities was exchanged to compensate the loss. Salinity was monitored regularly to ensure that evaporation did not affect its concentration during the trial period. Aquaria were checked periodically to determine fish movement after 24 h, 48 h, 72 h and 96 h. The same experiment was conducted in another set of aquaria for 24 h to determine loss of body weight and muscle tissue water. Fish became sluggish due to increasing salinity with time and the cessation of opercula movement was considered as criterion of fish death. The dead fish were removed and their numbers recorded.

2.4.3. Salinity resistance test

Six aquaria (each capacity 54 l) were filled with 50 l freshwater (0 ppt salinity). Then fish after 50 days of growth study were released @ 5 nos aquaria⁻¹ for salinity resistance test, each with triplicate. Mean weight and length of fish for T0 feed was 7.05 ± 0.03 g; 8.75 ± 0.03 cm and mean weight and length of fish for T1 feed was 9.50 ± 0.06 g; 9.07 ± 0.02 cm. Salinity of all 6 aquaria was increased (taken from stock solution) @ 1 ppt day⁻¹ until the salinity reached at 12 ppt. Two different feeds (T0 and T1) were applied separately in respective treatments (T0 feed for T0 treated fish and T1 feed for T1 treated fish) twice a day (10 AM and 16 PM) @ 3% of fish body weight. Water depth and test salinities were maintained throughout the experimental period. The fecal matter and left over feed were siphoned out daily. The dead fish were removed and their numbers recorded.

2.5. Sampling and calculation

2.5.1. Growth study

Fish sampling was done at every ten days interval to record their growth in terms of weight and length increment during freshwater experiment for 50 days. Individual fish were caught using scoop net from each tank of respective treatment during every sampling and blotted on absorbent paper to record their total weight using electronic balance (Acculab digital, India) and total length using scale (30 cm). Weight of fishes of each tank of respective treatment was then calculated separately, following the formulae as; Growth rate (% gain in body weight) = (Final weight – Initial weight)/Initial weight $\times$ 100; Specific Growth Rate (%) (average % increase in body weight per day) = $(\ln W_2 - \ln W_1) / (t_2 - t_1) \times 100$, Where, $\ln W_1$ = natural logarithm of the initial weight of fish at time t_1 , and $\ln W_2$ = natural logarithm of the final weight of fish at time t_2 ; Net increment = (Weight of fish at day 50 – Weight of fish at day 1); Average increment day⁻¹ = Net increment of weight / total no. of days; Feed Conversion Ratio (FCR) = feed given / weight gain of fish; Protein Efficiency Ratio (PER) = Increased body weight (g)/protein consumed (g); Body weight loss = body weight before exposure to respective salinity – body weight after exposure to same salinity.

2.5.2. Toxicity test on salinity

Toxicity test on salinity (MLS_{96 h}) as described by Peltier and Weber (1985) was calculated by pooling mortality data from replicates within treatments and LC₅₀ value for MLS_{96 h} was determined through Probit analysis using Microsoft Excel software following the Finney's methodology (Finney, 1952).

Loss of body weight was determined before and after 24 h exposure of fish to prescribed salinities (0 ppt, 3 ppt, 6 ppt, 9 ppt and 12 ppt) according to Gordon (1959). Then fish exposed to respective salinities were sacrificed by using MS222 as anesthetic to determine loss of muscle tissue water. Muscle tissue samples (150 to 500 mg) were taken

from the left side below the dorsal fin of fish. Care was taken to remove all skin and adhering scales. Samples were weighed immediately upon removal, then dried at 105 °C for 24 h and reweighed to determine percentage of water in the muscle tissue.

2.5.3. Water quality

The important physico-chemical parameters of water were measured with respective methods and related instruments, supported by references: temperature (mercury thermometer), pH (Digital pH meter), dissolved oxygen (Winkler's method), carbon dioxide (Potentiometric method, APHA, 2002), total hardness (Titration method, APHA, 2002) total alkalinity (Titration method, APHA, 2002) and salinity (Refractometer SIGNSTEK, England) were analyzed.

2.6. Statistical analyses

The mean growth increment, SGR, PER and FCR of *Labeo bata* were analyzed by employing student *t*-test (Snedecor and Cochran, 1967) and mean body weight loss and muscle tissue water content were analyzed by employing Two way ANOVA (Snedecor and Cochran, 1967) followed by Tukey post-hoc test.

3. Results

3.1. Growth increment and growth rate

Growth increment in terms of weight and length was higher in betaine fed fish than that in non-betaine fed fish (Table 2). Net increment of fish weight was 5.92 g in betaine fed (T1) fish and 3.48 g in non-betaine fed (T0) fish. Similarly, net increment of length was 3.140 cm in betaine fed fish as compared to 2.790 cm in non-betaine fed fish. Average weight and length increment day⁻¹ were respectively 0.1184 g and 0.0628 cm in betaine fed fish as compared to 0.0696 g and 0.0558 cm in non-betaine fed fish. Growth rate (%) exhibited (Table 3) that betaine fed fish showed significantly ($p < .05$) higher growth rate ranging between 16.79 ± 0.84 – 27.91 ± 3.33 as compared to growth rate between 13.58 ± 1.25 – 15.36 ± 0.29 by non-betaine fed fish throughout the experimental period. However, betaine fed fish obtained highest growth rate (27.91%) during 20 to 30 days, though it was not consistent throughout the experimental period.

T-test values for growth rate (%) in T0 were insignificant between the days 10–20, 20–30, 30–40, 40–50 and 10–50, whereas in T1 the values were significant ($p < .05$) between the days 10–20, 30–40 and 10–50. Comparison between T0 and T1 showed that values for growth (%) in all the days (10, 20, 30, 40, 50) were significantly higher ($p < .05$) in T1 than that in T0. *T*-test indicated that incorporation of betaine in feed (T1) exhibited higher growth rate (%) than that in non-betaine fed fish.

Table 2

Growth of *Labeo bata* fed with two formulated feeds (T0 and T1) during 50 days of rearing in freshwater.

Day	Average weight (g) day ⁻¹		Average length (cm) day ⁻¹	
	(T0)	(T1)	(T0)	(T1)
01	3.570 $\pm$ 0.00	3.580 $\pm$ 0.00	5.96 $\pm$ 0.03	5.93 $\pm$ 0.03
10	4.060 $\pm$ 0.05	4.340 $\pm$ 0.03	6.44 $\pm$ 0.04	6.47 $\pm$ 0.03
20	4.640 $\pm$ 0.02	5.550 $\pm$ 0.13	7.21 $\pm$ 0.02	7.24 $\pm$ 0.03
30	5.360 $\pm$ 0.03	6.860 $\pm$ 0.07	7.9 $\pm$ 0.02	7.96 $\pm$ 0.04
40	6.170 $\pm$ 0.03	8.140 $\pm$ 0.04	8.36 $\pm$ 0.02	8.58 $\pm$ 0.04
50	7.050 $\pm$ 0.03	9.500 $\pm$ 0.06	8.75 $\pm$ 0.03	9.07 $\pm$ 0.02
Net increment	3.480	5.920	2.790	3.140
Average increment/day	0.0696	0.1184	0.0558	0.0628

Values are in mean $\pm$ SD.

Table 3Growth rate (%) and student *t*-test values for growth rate of *Labeo bata* fed with two formulated feeds (T0 and T1) during 50 days of rearing in freshwater.

Days	Growth rate (%)		T-test values for growth rate				
	T0	T1	Between Days	T0	T1	Days	Between T0 & T1
10	13.58 ± 1.25	21.25 ± 0.69	10 to 20	0.975	3.393*	10	9.265*
20	14.47 ± 0.95	27.91 ± 3.33	20 to 30	1.563	1.969	20	6.73*
30	15.36 ± 0.29	23.64 ± 1.73	30 to 40	1.133	3.635*	30	8.174*
40	15.12 ± 0.22	18.68 ± 1.61	40 to 50	1.423	1.796	40	3.78*
50	14.31 ± 0.96	16.79 ± 0.84	10 to 50	0.796	7.079*	50	3.353*
AGR (%)	14.57	21.65					

AGR = Average growth rate (%); *t* 0.05, 4 = 2.77645.* Significant *p* < .05.

3.2. Specific growth rate (SGR), Feed conversion ratio (FCR) and Protein efficiency ratio (PER)

3.2.1. Specific growth rate (SGR)

Specific growth rate (SGR) is the most commonly used index used to measure growth. Cumulatively, betaine fed fish showed average 1.95% SGR which was significantly (*p* < .05) higher than average 1.36% SGR recorded in non-betaine fed fish throughout the experimental period (Table 4). The highest SGR (2.46%) was obtained in T1 fed fish during 20 to 30 days period. *T*-test was calculated for specific growth rate as i) between days and ii) between treatments. Result showed that the values between days (10–20, 20–30, 30–40, 40–50 and 10–50) in T0 were insignificant, whereas in T1 values as 3.450, 3.641 and 7.059 between 10–20, 30–40 and 10–50 days respectively were significant (*p* < .05). Even when the *t*-test was calculated between T0 and T1, the values in all the days (10, 20, 30, 40, 50) were significant (*p* < .05), and the specific growth rate (%) in T1 was higher than that in T0. *T*-test indicated that incorporation of betaine in feed (T1) exhibited higher specific growth rate than that in non-betaine fed fish.

3.2.2. Feed conversion ratio (FCR)

Overall, average FCR value as 0.950 of betaine fed fish was significantly (*p* < .05) lower than average FCR value as 1.619 observed in non-betaine fed fish throughout the experimental period (Table 5), with lowest FCR (0.786) recorded in T1 during 10 to 50 days. *T*-test calculated for FCR between days (10–20, 20–30, 30–40, 40–50 and 10–50) in T1 was not significant which indicated that incorporation of betaine in feed (T1) exhibited lower FCR than that in non-betaine fed fish. On the contrary, FCR in T0 was significant (*p* < .05) between days except the value in 40–50 days. However, FCR between the treatments T0 and T1 was significant (*p* < .05). *T*-test indicated that incorporation of betaine maintained lower FCR of fish as compared to that in non-betaine fed fish.

3.2.3. Protein efficiency ratio (PER)

Betaine fed fish (T1) showed average PER value as 3.687 which was

significantly (*p* < .05) higher than average PER value as 2.164 in non-betaine fed fish (T0) throughout the experimental period (Table 6), with highest PER value as 4.250 recorded in T1 during 10 to 50 days. *T*-test was calculated for PER between days in T1 was not significant which indicated that incorporation of betaine in feed (T1) exhibited higher PER than that in non-betaine fed fish. On the contrary, PER in T0 was significant (*p* < .05) between days except in 40–50 days. However, PER between the treatments T0 and T1 was significant (*p* < .05). *T*-test indicated that incorporation of betaine maintained higher PER of fish as compared to that in non-betaine fed fish.

3.3. Toxicity test on salinity

Non-betaine fed fish (T0) showed 20% mortality at 9 ppt after 72 h exposure and then showed 100% mortality at 12 ppt within 24 h (Table 7). When LC50 (Lethal Concentration 50) value for *MLS*_{96 h} was determined through Probit analysis (Finney, 1952), it showed 9.39 ppt as lethal salinity i.e., 50% of fish would die at salinity 9.39 ppt after 96 h. On the contrary, betaine fed fish (T1) showed no mortality even at 12 ppt after 96 h exposure.

3.4. Loss of body weight and muscle tissue water

In general, body weight declined with increasing salinity in both betaine (T1) and non-betaine fed (T0) fish. However, non-betaine fed fish showed cumulatively significant (*p* < .05) higher body weight loss (6.63%) compared to that in betaine fed fish (Table 8). Similarly, the amount of water in muscle tissue decreased about 1.0% with increasing salinity up to 9 ppt in both betaine and non-betaine fed fishes. But at 12 ppt, non-betaine fed fish showed significantly (*p* < .05) higher amount of water loss (11.6%), whereas betaine fed fish had insignificant water loss (1.3%) (Fig. 1). Over all, non-betaine fed fish lost 10.3% higher amount of water in muscle tissue as compared to that in betaine fed fish.

Table 4Specific growth rate (% body weight day⁻¹) and student *t*-test values for specific growth rate of *Labeo bata* fed with two formulated feeds (T0 and T1) during 50 days of rearing in freshwater.

Days	Specific Growth rate (%)		T-test values for growth rate				
	T0	T1	Between days	T0	T1	Days	Between T0 & T1
10	1.27 ± 0.11	1.93 ± 0.06	10 to 20	0.976	3.450*	10	9.115*
20	1.35 ± 0.08	2.46 ± 0.26	20 to 30	1.558	1.972	20	7.007*
30	1.43 ± 0.03	2.12 ± 0.14	30 to 40	1.133	3.641*	30	8.426*
40	1.41 ± 0.02	1.71 ± 0.14	40 to 50	1.426	1.803	40	3.838*
50	1.34 ± 0.08	1.55 ± 0.07	10 to 50	0.799	7.059*	50	3.356*
ASGR (%)	1.36	1.95					

Values are in mean (± S.D); ASGR = Average specific growth rate (%); *t* 0.05, 4 = 2.77645.* Significant *p* < .05.

Table 5Feed conversion ratio (FCR) and student *t*-test values for FCR of *Labeo bata* fed with two formulated feeds (T0 and T1) during 50 days of rearing in freshwater.

Days	FCR		T-test values for FCR				
	T0	T1	Between days	T0	T1	Days	Between T0 & T1
10	2.221 ± 0.20	1.411 ± 0.05	10 to 20	3.005*	7.655*	10	6.834*
20	1.830 ± 0.10	0.894 ± 0.11	20 to 30	5.251*	1.121	20	10.885*
30	1.502 ± 0.03	0.819 ± 0.04	30 to 40	8.664*	0.483	30	22.583*
40	1.323 ± 0.02	0.840 ± 0.06	40 to 50	2.436	1.303	40	12.892*
50	1.217 ± 0.07	0.786 ± 0.04	10 to 50	8.158*	18.347*	50	9.128*
AFCR	1.619	0.950					

Values are in mean (± S.D); AFCR = Average feed conversion ratio; *t* 0.05, 4 = 2.77645.* Significant *p* < .05.**Table 6**Protein efficiency ratio (PER) and student *t*-test values for PER of *Labeo bata* fed with two formulated feeds (T0 and T1) during 50 days of rearing in freshwater.

Days	PER		T-test values for PER				
	T0	T1	Between days	T0	T1	Days	Between T0 & T1
10	1.509 ± 0.08	2.365 ± 0.08	10 to 20	3.173*	5.441*	10	9.246*
20	1.825 ± 0.28	3.764 ± 0.12	20 to 30	6.164*	1.113	20	7.462*
30	2.219 ± 0.25	4.076 ± 0.22	30 to 40	9.170*	0.439	30	15.349*
40	2.520 ± 0.10	3.982 ± 0.02	40 to 50	2.251	1.259	40	8.223*
50	2.745 ± 0.07	4.250 ± 0.29	10 to 50	9.697*	15.014*	50	9.826*
APER	2.164	3.687					

Values are in mean (± S.D); APER = Average Protein efficiency ratio; *t* 0.05, 4 = 2.77645.* Significant *p* < .05.**Table 7**Survival (%) of *Labeo bata* fed with two formulated feeds (T0 and T1) during toxicity test on salinity for 96 h (MLS₉₆ h).

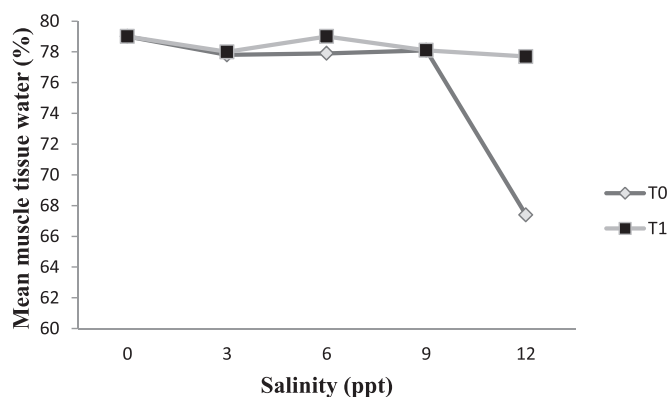
Salinity (%)	Survival rate (%)							
	T0				T1			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	100	100	100	100	100	100	100	100
3	100	100	100	100	100	100	100	100
6	100	100	100	100	100	100	100	100
9	100	100	80	80	100	100	100	100
12	0	0	0	0	100	100	100	100

3.5. Saline resistance test

Non-betaine fed fish (T0) started dying at 8 ppt with 10% mortality and eventually showed 90% mortality at 12 ppt (Fig. 2). However, they exhibited no mortality up to 7 ppt and then mortality occurred with each 1 ppt increase. On the contrary, 10% mortality started at 10 ppt in betaine fed fish (T1) and eventually 50% mortality at 12 ppt with each 1 ppt increase.

Table 8Average body weight (g ± SD) of *Labeo bata* fed with two formulated feeds (T0 and T1) after 24 h exposure to different salinities.

Salinity (%)	T0				T1			
	Body wt. before exposure	Body wt. after exposure	Body wt. loss	Body wt. loss (%)	Body wt. before exposure	Body wt. after exposure	Body wt. loss	Body wt. loss (%)
0	7.65 ± 0.26	7.57 ± 0.28	0.08 ± 0.04	1.1 ^a	9.10 ± 0.58	9.04 ± 0.58	0.06 ± 0.01	0.70 ^a
3	7.69 ± 0.52	7.54 ± 0.52	0.15 ± 0.03	2.0 ^a	8.06 ± 0.39	7.98 ± 0.39	0.08 ± 0.02	1.02 ^b
6	7.49 ± 0.90	7.27 ± 0.89	0.22 ± 0.04	3.0 ^{ab}	8.51 ± 0.73	8.31 ± 0.73	0.20 ± 0.02	2.37 ^c
9	6.54 ± 0.45	6.24 ± 0.47	0.31 ± 0.03	4.9 ^{bc}	8.52 ± 0.67	8.30 ± 0.67	0.23 ± 0.02	2.71 ^d
12	7.44 ± 0.58	6.96 ± 0.59	0.48 ± 0.05	6.9 ^c	8.72 ± 0.65	8.33 ± 0.68	0.39 ± 0.05	4.70 ^e

Values with same superscript do not differ significantly (*p* < .05), Values are in mean ± SD.**Fig. 1.** Mean muscle tissue water (%) of *Labeo bata* fed with two formulated feeds (T0 and T1) after 24 h exposure to different salinities.

3.6. Water quality parameters

During the experimental period (50 days), the water temperature ranged from 20 to 26.6 °C, pH from 7.5 to 8.1, dissolved oxygen from

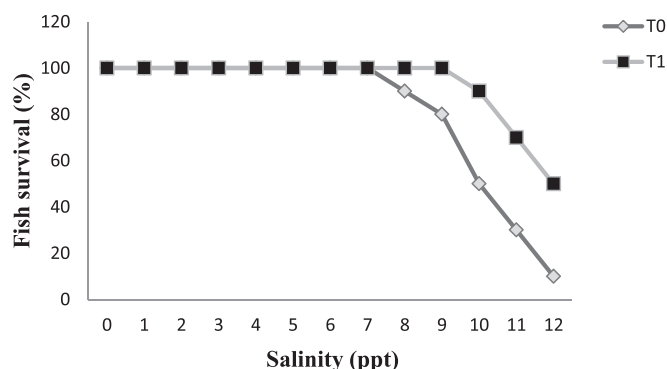


Fig. 2. Survival (%) of *Labeo bata* fed with two formulated feeds (T0 and T1) after gradually increasing salinity (@ 1 ppt day⁻¹) during 12 days of salinity resistance test.

5.62 to 6.67 mg l⁻¹; free carbon dioxide from 4.12 to 4.92 mg l⁻¹; total alkalinity from 160.20 to 163.28 mg l⁻¹ and total hardness from 820.33 to 970 mg l⁻¹.

4. Discussion

Labeo bata fed with the betaine supplemented feed showed higher growth rate, SGR and PER as compared to that in non-betaine fed *Labeo bata*. Betaine prevents enzyme inhibition, stimulates digestive enzyme activity to promote higher feed consumption (Srinivasa, 2000) and thereby plays an important role in tissues as a methyl donor in protein and energy metabolism (Virtanen and Soivio, 1985). Evidently, betaine promotes feed intake and enhanced growth rates in sea bass, *Dicentrarchus labrax* (Dias et al., 1997; Brotons et al., 2004), striped bass, *Morone saxatilis* (Papatryphon and Soares, 2000, 2001), Gibel carp, *Carassius auratus gibelio* (Xue and Cui, 2001), Tilapia, *Oreochromis niloticus* (El-Husseiny et al., 2008), Common carp, *Cyprinus carpio* (Murthy et al., 2016), and Prawn, *Macrobrachium rosenbergii* (Pavadi et al., 2012). Betaine acts as a powerful attractant and inducer of enhanced feeding behavior with following evidences: *Macrobrachium rosenbergii* fed with glycine betaine-added feed shows higher feed intake and lower FCR as compared to controlled one (Felix and Sudharsan, 2004), Nile tilapia, GIFT strain fed with a betaine feed @ 5 g kg⁻¹ accounts lower FCR (Luo et al., 2011), *Labeo rohita* fed with 0.25% betaine shows lower FCR than that in normal one (Shankar et al., 2008) and *Morone saxatilis* fed with both levels (2% and 4%) of the FS mixture (L-alanine, L-serine, inosine-5-monophosphate and betaine) shows significantly lower FCR compared to that of fish fed the basal feed (Papatryphon and Soares, 2000).

Betaine is an organic osmolyte and acts as osmoprotectant to control the regulation of the intracellular osmotic balance in fishes. Thus, betaine fed fish was able to maintain their muscle tissue water balance higher than that in non-betaine fed fishes during osmotic stress (Daikoku, 1980). Betaine fed fish is also able to prevent body weight loss during osmotic stress, because betaine can control osmoregulation by preventing abnormal water loss from the cells against dramatic changes in osmotic pressure in fish (Clarke et al., 1994). It accumulates in muscle cells to enable fish acclimatized in changing salinities and thereby increases eating ability (Clarke et al., 1994; Castro et al., 1998). In the present experiment, water content of the muscle of betaine fed *L. bata* lost only 1.3% from freshwater to 12 ppt salinity due to effect of betaine as compared to 11.6% water loss in the muscle of non-betaine fed *L. bata* in the same salinity.

The survival rate of betaine fed fish was 50% as compare to 10% survival rate of non-betaine fed fish at 12 ppt. Betaine fed fish could maintain its normal physiological balance during osmotic stress, whereas non-betaine fed fishes failed to check dehydration and showed poor survival. Higher survival rate of betaine fed fish was possible due

to addition of betaine, because absorbed betaine acts as an osmolyte within fish body and reduces the osmotic stress in fish (Castro et al., 1998) through preventing abnormal water loss. Also, Hoseini and Hosseini (2010) supported that betaine fed common carp group showed no mortality until 168 h as compared to controlled ones with near 100% mortality at 72 h after osmotic challenge.

The water quality parameters recorded during the experimental period were found suitable and well within the range suitable for *Labeo bata* culture (Jhingran, 1991). Though hardness of the water was found high, which might have caused the fish growth disturbed. Further, it was also observed that the incorporation of betaine in feed did not affect the tank water.

5. Conclusion

Evidently, dietary supplementation of betaine improves growth, PER, FCR and salinity tolerance limit in *Labeo bata*. The present study suggests that incorporation of betaine in supplementary feed may enable fishes to survive up to 12 ppt when saline water happens to ingress in freshwater environment. In south Asian countries, freshwater areas of coastal regions are vulnerable to have saline water ingress due to extreme climatic situations including tidal flood, sea waves, tidal thrust, cyclone, and tsunami. In such condition, cultivation of freshwater fish species may be at stake and farmers may have a substantial economic loss. However, this preliminary study may encourage farmers to cultivate *Labeo bata*, a freshwater species, in saline environment after treated with betaine added feed.

Acknowledgements

The authors are thankful to Head of the Department of Aquaculture and field staff of the Faculty of Fishery Sciences, Kolkata for their help in carrying out the study.

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